Preliminary report for The Type Approval Test

Used by Electro-Cleen™ Ballast Water Management System

March 2008

South Sea Research Institute
Korea Ocean Research & Development Institute

TEST REPORT

391 Jangmok-Ri Jangmok-Myon Geoje, 656-830, KOREA TEL: +82 55 639 8400 FAX: +82 55 639 8419
Homepage : http://www.kordi.re.kr/ssi

Title

PRELIMINARY REPORT FOR THE TYPE APPROVAL TEST USED BY ELECTRO-CLEAN BALLAST WATER MANAGEMENT SYSTEM

Customer TECHCROSS Inc.	**Project No.** PI49300
Administration Ministry of Maritime Affairs & Fisheries, Republic of Korea	**Project Manager** Shin, Kyoungsoon
Test Items Land-Based Test and Shipboard Tests According to Guidelines for Approval of Ballast Water Management Systems(G8) of IMO	**Date** 3rd March 2008

Summary :

Land-based and shipboard tests as a type approval test for the Electro-Clean Ballast Water Management System are currently being conducted according to the Guidelines for Approval of Ballast Water Management Systems(G8) and Procedure for Approval of Ballast Water Management Systems that Make Use of Active Substances(G9). For the application of final approval of a ballast water management system using active substances, preliminary results are reported to the Government and Customer. All of the tests are conducted using the quality system of ISO/IEC 17025 based on the Provisional Regulation of Type Approval of Ballast Water Management System.

Project Manager : _____

Shin, *K*youngsoon

Contents

1. Introduction

The South Sea Institute of KORDI is the operating laboratory that is accredited by KOLAS (The Korea Laboratory Accreditation Scheme) for ISO/IEC 17025 in field of aquatic organisms and all efficacy tests are conducted by the quality system of ISO/IEC 17025. KOLAS is the governmental accreditation body in the Republic of Korea. KOLAS evaluates the technical competence of testing and calibration laboratories based on the general requirements of ISO/IEC 17025, general requirements for the competence of testing and calibration laboratories and specific technical requirements of each field (Fig. 1).

Fig. 1. South sea institute of KORDI

The efficacy of ballast water management systems which produce active substances was evaluated by administration in accordance with guidelines for approval of ballast water management systems (G8). The guidelines (G8) include general requirements concerning design and construction, technical procedures for evaluation and the procedure for issuance of the Type Approval Certificate of ballast water management system. The guidelines (G8)

are unique compared to the requirements for type approval of traditional marine equipment as type approval of ballast water treatment systems requires both land-based and shipboard test.

The procedure and conditions of land-based and shipboard tests are as such in Fig. 2 and 3.

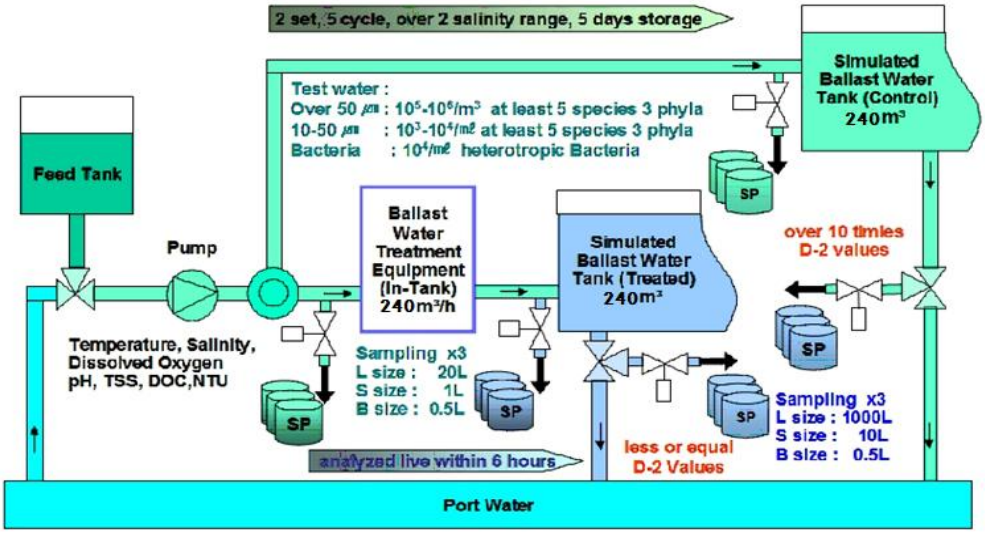


Fig. 2. Land-based test procedure and conditions

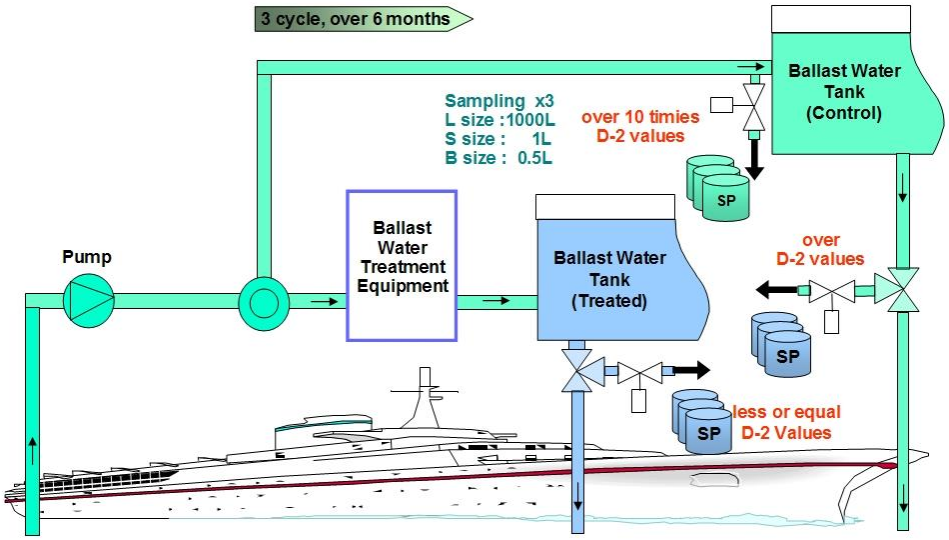


Fig. 3. Shipboard test procedure and conditions

(ISO/IEC 17025:1999(E))

2. Land-based test

The land-based tests were performed at two different sites with more than 10PSU difference in salinity according to the G8 guidelines. The locations of the land-based tests were Masan Bay at South Sea of the Korean Peninsular for seawater, and down stream of the Sumjin River in the southern part of the Korean Peninsular for brackish water (Fig. 4).

The Korea Ocean Research and Development Institute performed all of the tests that took place, and observed D-2 regulations

The tests in Masan Bay for seawater were performed 5 times from June 2007 to January 2008. The tests in the Sumjin River for brackish water were performed 6 times from August 2007 to February 2008. The last test in Sumjin River was carried out for the analysis of disinfection by-product according to the D-2 standard.

The working standard of ECS was set at TRO concentration of 10mg/L. The electric current and voltage were varied in relation to the quality of environments in which the tests took place (salinity, temperature, and contents of organic matter, etc).

The land-based test was performed successfully five times for sea water and six times for brackish water respectively. The result for the disinfection efficacy test of brackish water was satisfying D-2 standard. However, the population densities of micro-organism between 10~50 μm were insufficient due to the seasonal changes in the Korean brackish water. Therefore micro-organisms were cultured to meet its initial concentration.

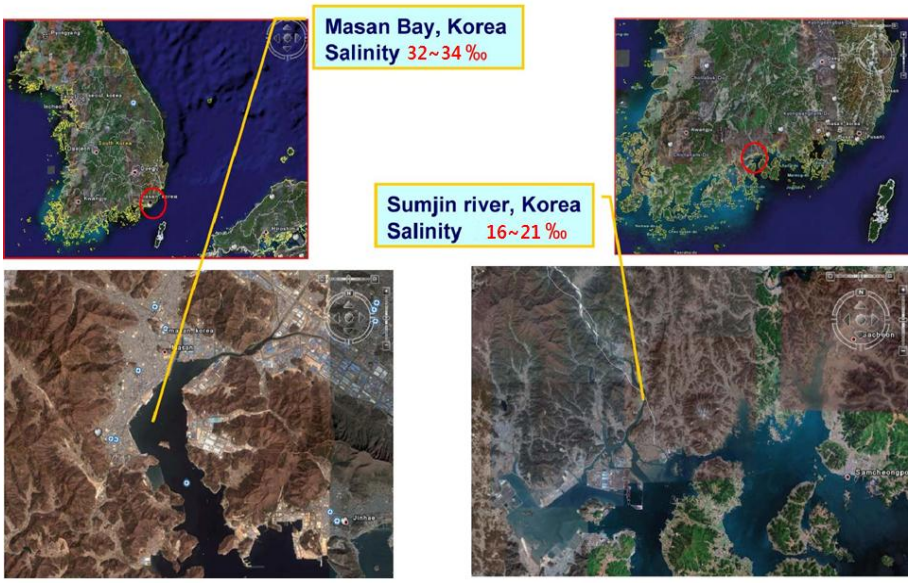


Fig. 4. Sites for land-based tests

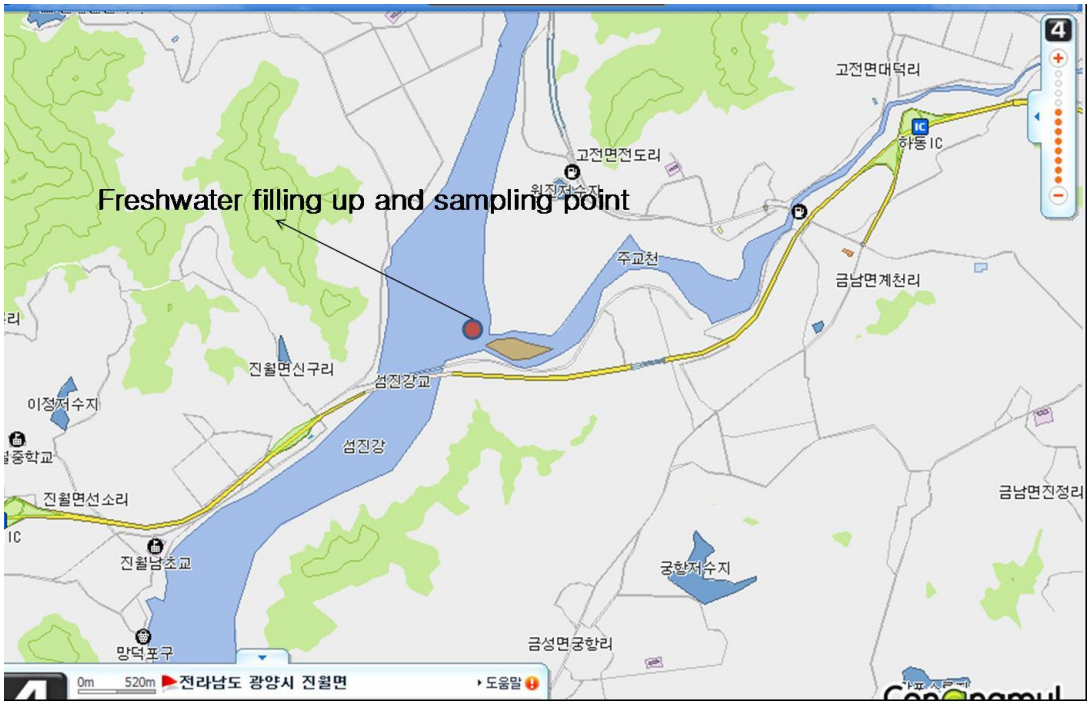


Fig. 5. Freshwater sampling point

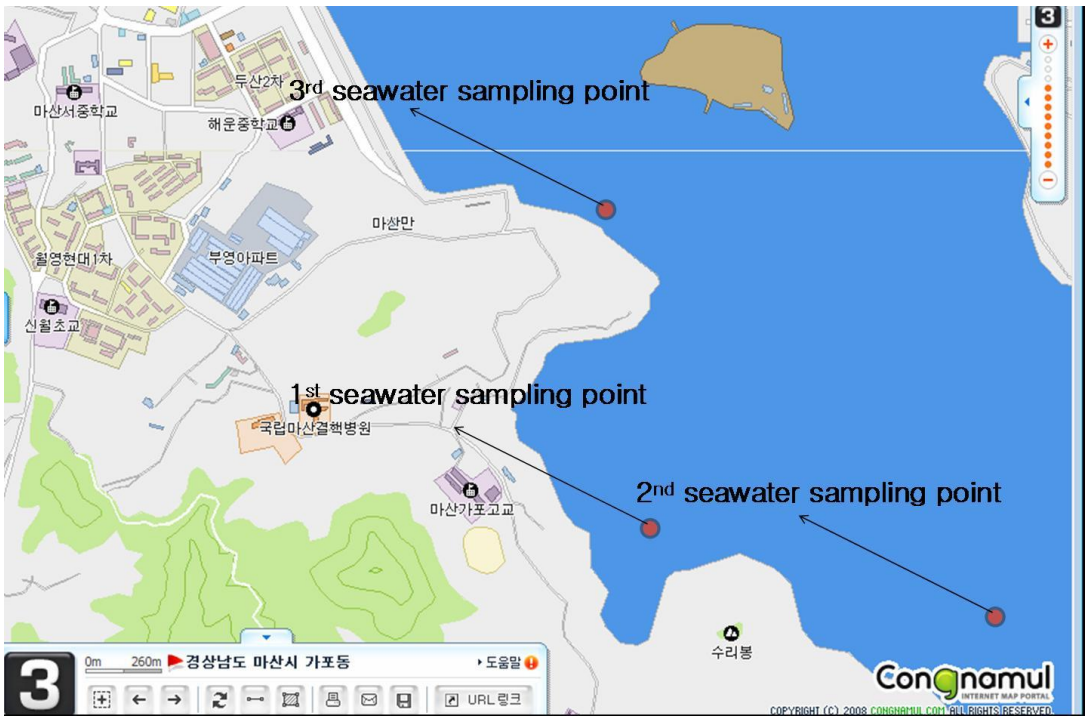


Fig. 6. Seawater sampling point

2.1. Information of test barge

Four test tanks were built for the barge, the ECS-300A was installed for the land-based test, and the safety, application and ecological efficacy of the ECS was proved.

Each of the four test tanks has a volume of 240m^3 (Tables 1 and 2). All pipes and valves in each test tank were designed and installed based on the regulation, and four ballast pumps sized 500m^3/h were installed. For sampling, four tanks for treated water which are 2m^3 in volume and the four controlled water tanks with pipelines and valves were installed.

Table 1. Barge for ECS land-based test specification

Item	Specification
Name / Type	ELECTRO CLEAN/ Barge
StorageTank	Four Tanks of 240 m^3
Dimensions	Length 34.31m Width 13.40m Depth 2.4m
Builder	Daeun Shipyard
Launching Date	May 1, 2006

Table 2. Features of the barge

Item	Description
Pipes & pumps facilities	Maximum pump capacity 2,000m^3/h (4 × 500m^3/h)
Lightproof tank facilities	Two 240m^3 class simulated ballast tanks Two 240m^3 class culture tanks Eight 2m^3 test tanks
Sampling facilities	Electric netting equipment for micro-organism criteria Sample concentrator Sampling net
Micro-organism cultivation facilities	Microscope lab Micro-organism disposal chamber Sample testing lab NaOCl elimination agent feeder
Power generating facilities	3 power generators for operation (Total 800-kw class)
Ballast management system	ECS-300A & extra equipment
Maintenance tools	Spare parts and tools for maintenance
Other facilities	Accommodations for researchers and staff (Dining room, bedroom, conference room)

2.1.1. Advantage of barge for the land-based test

- It is difficult to control the population density of micro-organisms according to D-2 regulation when the test is operated as it is limited to a land-based location. However, it is straightforward to choose the preferred location where the population density of micro-organisms for the test can be controlled.
- Temperature inside the ballast tank can be kept at the same level as that of the sea, so that the culture conditions for the test micro-organisms can be maintained as same as the micro-organisms outside the ship.
- It is possible to have many different water conditions for the pre-test before the actual land-based test.

- The instrument to create the active substance requires the same level of electrical power as that of actual ships in order to design the specific instrument.
- For the removal efficacy of micro-organisms when tested in sea regions where POC concentrations and salinity are different to each other, the cost for re-installation of the facility is reduced.

2.1.2. Specifications of electrolyzer

The components of ECS can be summarized as follows (Table 3):

- Product name: ECS (Electro-Clean System)
- Model: Electrolyzer module (ECS-300A)
- Usage: Ballast Water Management System (BWMS)
- Capacity: 300m^3/h (Ballast pump) Max
- Disinfection method: Disinfection by electrochemical oxidation
- Disinfection efficacy: Compliance with D-2 Regulation of IMO
- Active substances dosage: TRO (Total Residual Chlorine) 10mg/L ± 20% Nominal (in electrolysis module)
- Maximum disinfection performance: TRO 12mg/L ± 20% Max (in electrolysis module)
- Operating mode: Manual/ Automatic mode via computer control
- Operating software: ECS-2000 (OS: Window NT, XP or above)
- Power consumption: 18kW/h or nominal
- Operating temperature: 0~55℃ (Installed in a closed and environmentally controlled space)

Table 3. ECS system components

Component	Device	Unit quantity	Model
Control unit	Control PC	1EA	ECS-CPC
	R T U (Remote Terminal Unit)	1EA	ECS-RTU
Power Distributor Unit	Power Distributor	1EA	ECS-EPDS
	Multifunctional relay (H I M A P)	1EA	ECS-HIMAP
Rectifier Unit	Rectifier (TC15D3KS)	1EA	ECS-Power
	Cooler (TECU50)	1EA	ECS-Cooler
Electrolysis Unit	Electrode	4EA	ECS-EM
	Chamber	1EA	ECS-EMC
Operation S/W	HMI (S/W)	1EA	ECS-OS
Sensor Unit	TRO sensor (Online)	1SET	CLX (Maker: HF Scientific, inc.)
	TRO sensor (Online, option)	1SET	CL7685.10 (Maker: B&C)
	PH sensor (Online, option)	1SET	PH7685.010 (Maker: B&C)
	Conductivity sensor (Online, option)	1SET	C7685 (Maker: B&C)

2.2. Installation and sampling procedure on the barge

The barge is composed of four 240m^3 tanks and ECS-300A module that is capable of treating 300m^3 of ballast water per hour with each pipe and valve fitted to the right standard and four 500m^3/h ballast tanks to take up sea water (Fig. 7). A blower system is installed for the cleaning of the sampling tanks. There are 4 tanks of 2m^3 to control the dosage and each tank has an attached output valve to allow the possibility of sampling.

The description of the barge and sampling procedure are as follows (Fig. 8 and Table 4):

Fig. 7. Description of barge for land-based test

Fig. 8. Side view of barge

Table 4. Sampling procedure

Procedure	Water Flow	Sampling
Uptake form sea chest	Strainer → Pump → Tank 2, Tank 3	
Uptake form tank and/or sea chest, treatment and store at treated and control tank	Tank 2 and/or Tank 3 → Pump → (Parallel or Sequential) w/ Treatment → Tank 4 w/ Treatment → Tank 1	Before Treatment: 20 liter × 3 times Before Tank 4: $1m^3$ × 3 times Before Tank 1: 20 liter × 3 times
Discharge of treated water	Tank 4 → Pump → Outlet	Before Outlet: $1m^3$ × 3 times
Discharge of control water	Tank 1 → Pump → Outlet	Before Outlet: $1m^3$ × 3 times

2.3. Preparation for the test

2.3.1. Cleaning tanks and pipes

The ballast water tanks in the barge were cleaned by fresh seawater pumped from the sea to remove dirt and residues left over in the tanks. And also 10 minutes before each test takes place, water from fresh water tanks is pumped into each ballast water tank in order to remove any leftovers from polluted water.

2.3.2. Test instruments

If the surface of any electronic instruments such as: rectifier, power distributor, and control PC gets contaminated, they should be cleaned by a clean fabric and cleaner. Any rusted instruments should be replaced. Any instrument with an air-filter should also be replaced. Before the test, if the electrode and cable line of ECS module are rusted, it should be cleaned by rust remover and protected by liquid coating.

2.3.3. Test water

Test waters were prepared in a 480 m^3 tank using high salinity sea water at Masan Bay or brackish water at the Sumjin Estuary depending on the required salinity (> 32 PSU or 3-32 PSU, respectively, with a minimum difference of 10 PSU). The 480 m^3 of test water was used for both testing and control.

The combination of harvested indigenous organisms and cultured surrogate species (> 50μm: *Artemia salina*, 10-50μm: *Amphidinium carterae, Heterocapsa triquetra, Prorocentrum minimum, Scrippsiella trochoidea Tetraselemis* spp., and/or *Thalassiosira* spp.) was added to fulfill the biological water quality criteria. One or more cultured species were used at a time depending on the abundance of harvested indigenous organisms and culture conditions. Also, soluble lignin, starch (or seaweed powder) and kaolin (or prepared mud) were added to adjust the initial concentrations of dissolved organic carbon (DOC), particulate organic carbon (POC) and total suspended solids (TSS) to meet chemical water quality criteria.

2.4. Test methods

All nets for the concentration of organisms in the test site should be strictly separated into three sets (each set uses 2 types of net; 7μm mesh and 45μm mesh in diagonal dimension) to avoid cross-contamination.

2.4.1. Temperature and salinity

Temperature and salinity were measured using an Idronaut Ocean Seven 319 CTD. The sensing unit was calibrated once a year by the manufacturer.

2.4.2. pH

A pH meter used was a model 1230, manufactured by Orion Research Inc. The meter was calibrated using standard solutions (Orion application buffer, pH 4, 7, 10) each time prior to the measurement according to the guidance provided by manufacturer.

2.4.3. Dissolved oxygen

DO was measured using an Idronaut Ocean Seven 319 CTD. The DO meter attached to the CTD was calibrated prior to the measurement according to the guidance provided by manufacturer. Maintenance (mostly membrane and electrolyte replacement) should be carried out at least every three months.

2.4.4. Determination of dissolved organic carbon (UNESCO, 1994)

For dissolved organic carbon (DOC) analysis, each seawater sample was filtered through a pre-combusted (450 °C, 2hours) 25mm Whattman GF/F filters (nominal pore size 0.7 μm) and 20 mL of filtered sample was collected in a pre-combusted 30-mL EPA vial (Wheaton, WH.W227354). After collection, the filtered seawater sample was immediately acidified with 10% H3PO4 solution and purged with ultrapure O_2 gas for 10 min to remove dissolved inorganic carbon (DIC). One hundred microliters of the DIC-free subsample was then injected into the combustion tube of total organic carbon analyzer Shimadzu TOC-VCPH for the oxidation of DOC to CO_2, which was facilitated by a platinum catalyst at 650 °C. The liberated CO_2 was subsequently measured using an infrared detector. On each day measurements were performed, a three-point calibration curve was constructed using potassium phthalate standards freshly prepared in Milli-Q water. These standards covered a DOC concentration ranged of 0 to 10 mg/L and were run once a day. All DOC measurements reported here represent the mean of three injections from each sample.

2.4.5. Determination of dissolved particulate organic carbon (UNESCO, 1994)

For determination of dissolved particulate organic carbon (POC), samples were collected in 2 liter sterilization bottles. 200 mL of seawater sample was filtered onto pre-combusted (450 °C, 2hours) 25mm Whattman GF/F filters (nominal pore size 0.7 μm). During filtration,

a slight vacuum (0.0027 MPa or 200 torr) was applied to avoid rupture of the cells on the filters. After filtration, the wet filters were dislodged from the filter holders and then stored frozen in a deep freezer (-20 °C) until processed. Prior to analysis, the filters are placed overnight in a desiccator saturated with HCl fumes. The air in the desiccator is kept saturated by leaving concentrated HCl in an open container in the lower compartment of the desiccator. Thereafter, the filters are dried again at 65 °C for two days. Immediately before analysis, with the use of a clean pair of forceps, the dried filters were folded with tinfoil and palletized (Sharp, 1974). The CHN elemental analyzer (ThermoQuest, EA1112) was used to determine the concentration of POC on the dried filters.

2.4.6. Determination of total suspended solids (APHA, 1995)

Whattman GF/F filters (47mm, nominal pore size 0.7 μm) were rinsed with 20 mL of distilled water three times and dried in the oven at 103 to 105 °C for 1 hour and then cooled to the room temperature for 30 minutes in the desiccator. The filters were then weighted. The sample volumes were chosen for the sufficient amount to yield residue between 10 mg and 200 mg after filtration. When there were very low total solids in samples (less than 10 mg/L), the deficiency of low weight was compensated by using a high-sensitive balance (0.001 mg). The appropriate amount of sample was filtered through the prepared 47mm Whattman GF/F filter and the residue retained on the filter was dried at 103 to 105 °C for 1 hour. The filter was cooled in desiccators to room temperature for 30 minutes, and then re-weighted. The difference between pre and after weighted filter was divided by sample volume (unit liter) and represents the concentration of total suspended solid.

2.4.7. Viability test of organisms

- **Organisms ≥ 10~50μm**

  The disinfection efficacy of the ECS on 10~50μm sized organisms (mainly phytoplankton) was assessed by three kinds of measurements using light microscope, epifluorescence microscope and fluorometer (Turner Designs 10-AU).

  With the use of the light microscope, the motility; for example, sliding or its own original movement is considered as an indication of vitality of the phytoplankton.

  Chlorophyll autofluorescence is used as an indicator of cell viability (Pouneva, 1997). Intact chlorophyll of living cells shows red fluorescence, while dead or severely damaged

chlorophyll lose the red fluorescence. Commonly, under a green filter using an epifluorescence microscope, most of the living cells show bright red colour, while the dead cells show faintly green colour or disappearance of red fluorescence.

Fluorometer with high sensitivity can detect the fluorescence of phytoplankton more than one live cell per milliliter. Thus, if no value is read on the display of fluorometer, it means that all cells are considered to be 'dead', while it means that there are live cells in the sample if any number is read. Fluorometer was used as a supplementary means. Also, the applied method was fluorescence diacetate (FDA) staining to assess viability of 10~50μm sized organisms.

FDA stain: The FDA stock solution was prepared by mixing with regent grade dimethylsulfoxide (DMSO) at a concentration of 5 mg/mL following the instructions of Gervey *et al.,* (2007). FDA was commercially available from Sigma Co. This stock solution was stored in a refrigerator at 7℃ (DMSO freezes at this temperature) and was thawed each day for the preparation of a working solution (Jochem 1999). A working stock solution was prepared by diluting the primary DMSO solution 100 times with chilled distilled water (50 μg/mL). The solution was mixed during preparation to prevent the precipitation and kept cold in the dark. Each sample was stained by adding 100 μl of the working solution to 3 mL sample (end concentration: 1.7 μg mL^{-1} FDA). Stained samples were kept in cool and dark place for a minimum of 10 minutes prior to enumeration. Samples could be saved for up to 90 minutes without risking significant fluorescent degradation if kept in the dark and in an ice-bath.

Viability counts using FDA: The enumeration using epifluorescence microscopy to count FDA stained phytoplankton cells was described by Gervey *et al*., (2007). The fraction of FDA-stained cells (viability) was determined under an epifluorescence microscope at 100× to 400× magnification, depending on cell size. Microplanktonic organism, which showed only green-fluorescence (wavelengths 520 to 530 nm) and did not emit red autofluorescence without a mobility, were counted under blue light excitation (wavelengths 450 to 500 nm) as viable organisms, which were non-autotrophic, except where stated otherwise.

- **Organisms > 50 μm**

Survivorship of the larger organisms 50μm (mainly zooplankton) was determined based on the appendage's movement under a stereomicroscope (APHA-804C, 1985). In each taxonomic group, individuals were classified as live or dead and counts of each group

were recorded. Animals were considered to be 'live' if they are actively moving or exhibited an escape behavior when probed with a fine needle. If no activity or movement of any kind was observed, after the additional sticking with a fine needle, the animals were considered to be 'dead'. And live or dead determination is confined to the unimpaired body of zooplankton. The assessment method mentioned above was also applied to the non-motile organisms larger than 50 μm.

- **DAPI count for heterotrophic bacteria**

For setting up the condition of influent water, 50mL seawater was fixed with neutralized formalin (final conc. 2~4%). One millilitre in well-fixed seawater is stained with 0.2mL DAPI (6-diamidino-2-phenylindole) working solution. After 5 minutes, at least 10 fields were counted under UV filter set of fluorescent microscopy at (× 1,000).

- **Indicator microbes**

• *Escherichia coli* (*E. coli*)

A 10mL of sea water was filtered onto the 0.2μm membrane filter and then filters are placed on the top of *E. coli*/ Coliform and Coliform Count Plates (3M^{TM} Petrifilm plate). The Petrifilm plates were incubated for 24 hrs at 35℃. The blue to red-blue colonies associated with entrapped gas in the Petrifilm EC plate (within approximately one colony diameter) is considered as *E. coli*. The enumeration of each sample was repeated eight to ten times.

• *Vibrio cholerae* (serotypes O1 and O139)

5 to 10 mL of sea water was filtered onto the 0.2μm membrane filter and then filters were placed on the TCBS (Bisulfate Citrate Bile Sucrose) agar. Pre-treated TCBS agar plates were incubated for 24 hrs at 35℃. The green coloured CFU is considered as *Vibrio parahaemolyticus*. The yellow coloured CFU is isolated into nutrient agar and incubated for 24 hrs at 35℃. If the cultivated CFU has purple colour, it is considered to be positive, if not or partly purple, it is negative. When the incubated CFU is decided as positive, API20E test should be carried out. The possibility of *Vibrio cholerae* in the case of partly purple coloured CFU was tested, but any CFU was not found in the API20E test. The enumeration of each sample was repeated eight to ten times.

• ***Enterococci***

A 20 ~ 40 mL of sea water was filtered onto the 0.2μm membrane filter and then filters are placed on the Intestinal *Enterococci* agar plate. The pre-treated agar plates were incubated for 48 hrs at 35℃. The pink to brown coloured CFU with a diameter of 0.5 to 2 mm is usually *Enterococci*. The enumeration of each sample was repeated eight to ten times.

Finally, the test mentioned above was additionally conducted with disinfected distilled water in order to confirm whether there was any contamination as a control or not.

Water quality of each test is summarized in Table 5 and 6.

Table 5. A brief summary of water qualities at the test waters for land-based test on sea water with > 32PSU (Masan Bay)

	S-1	**S-2**	**S-3**	**S-4**	**S-5**
Salinity (PSU)	33.4	33.2	32.7	32.1	32.9
DOC (mg/L)	1.6	1.7	1.6	1.7	1.8
POC (mg/L)	3.3	10.1	2.9	2.0	1.1
TSS (mg/L)	48.8	90.3	40.4	63.1	58.5

Table 6. A brief summary of water qualities at the test waters for land-based test on brackish water with 3~32PSU (Sumjin Estuary)

	B-1	**B-2**	**B-3**	**B-4**	**B-5**	**B-6**
Salinity (PSU)	19.3	18.2	18.6	19.2	20.1	16.8
DOC (mg/L)	5.4	6.7	6.5	5.7	6.1	5.4
POC (mg/L)	6.2	6.0	5.5	6.3	5.8	6.1
TSS (mg/L)	52.9	60.3	60.3	63.5	57.9	59.5

2.5. Test results

2.5.1. S-1 (Sea water in Masan Bay)

The organism concentrations of test water for the land-based test sampling were set following the guideline of G8. The water quality of the location is summarized in Table 7. The range of the organism concentration for the group bigger than 50μm in size was from 265,440 to 273,000 inds/m^3, while that of the group between 10μm and 50μm in size was from 1,253 to 1,443 inds/mL, in test water (Table 8). The range of heterotrophic bacterial population density was from 2.4 to 3.0 $\times 10^6$ inds/mL in test water (Table 8). However, after ECS treatment, the number of living organisms for all groups in control and treated water met the D-2 regulation.

Table 7. Water quality of sea water (Masan Bay)

Date	Sample	pH	Temp. (°C)	Salinity (PSU)	DO (mg/L)	TSS (mg/L)	DOC (mg/L)	POC (mg/L)
2007.06.06	**Test water**	8.0	19.8	33.4	6.9	48.8	1.6	3.3
2007.06.11	**Control**	8.0	22.0	33.1	5.6	67.8	1.3	4.3
2007.06.11	**Treated**	7.9	21.5	33.1	7.1	71.9	1.8	2.2

Table 8. Biological efficacy of ECS from land-based test at sea water (Masan Bay)

Date	Sample	Sampling at	50μm< (inds/m^3)	10~50μm (inds/mL)	Heterotro. Bacteria (inds/mL)	*E. coli* (cfu/100 mL)	*Vibrio cholerae* (cfu/100 mL)	*Entero coccus* (cfu/100 mL)
2007.06.06	**Test water**	**20%**	265,440	1,321	3.0E+06	68	0	65
		50%	272,630	1,443	3.0E+06	133	0	192
		80%	273,000	1,253	2.4E+06	36	0	130
2007.06.11	**Control**	**20%**	1,238	208		15	0	10
		50%	2,554	69		55	0	45
		80%	1,661	277		10	0	30
2007.06.11	**Treated**	**20%**	0	3		0	0	0
		50%	0	4		0	0	0
		80%	0	3		0	0	0

2.5.2. S-2 (Sea water in Masan Bay)

The organism concentrations of test water for the land-based test sampling were set as the guideline of G8. The water quality of the location is summarized in Table 9. The range of the organism concentration for the group bigger than 50μm in size was from 502,499 to 704,700 inds/m^3, while that of the group between 10μm and 50μm in size was from 1,942 to 2,542 inds/mL in test water (Table 10). The range of heterotrophic bacterial population density was from 3.4 to 4.3 $\times 10^6$ inds/mL in test water (Table 10). However, after ECS treatment, the number of living organisms for all groups in control and treated water met the D-2 regulation.

Table 9. Water quality of sea water (Masan Bay)

Date	Sample	pH	Temp. (℃)	Salinity (PSU)	DO mg/L	TSS mg/L	DOC mg/L	POC mg/L
2007.06.20	**Test water**	8.2	20.5	33.2	7.0	90.3	1.7	10.1
2007.06.25	**Control**	7.9	22.1	33.2	2.7	97.2	1.7	5.8
2007.06.25	**Treated**	8.1	22.0	33.2	6.3	94.2	2.5	3.8

Table 10. Biological efficacy of ECS from land-based test at sea water (Masan Bay)

Date	Sample	Sampling at	50μm< (inds/m^3)	10~50μm (inds/mL)	Heterotro. Bacteria (inds/mL)	*E. coli* (cfu/100mL)	*Vibrio cholerae* (cfu/100 mL)	*Entero coccus* (cfu/100mL)
2007.06.20	**Test water**	**20%**	502,499	2,330	3.5E+06	62	0	49
		50%	704,700	1,942	3.4E+06	107	0	88
		80%	526,080	2,542	4.3E+06	107	0	46
2007.06.25	**Control**	**20%**	3,708	40		15	0	5
		50%	5,191	160		20	0	15
		80%	8,702	110		25	0	10
2007.06.25	**Treated**	**20%**	0	3		0	0	0
		50%	0	1		0	0	0
		80%	0	1		0	0	0

2.5.3. S-3 (Sea water in Masan Bay)

The organism concentrations of test water for the land-based test sampling were set as the guideline of G8. The water quality of the location is summarized in Table 11. The range of the organism concentration for the group bigger than 50µm in size was from 233,492 to 267,733 inds/m^3, while that of the group between 10µm and 50µm in size was from 1,608 to 2,077 inds/mL in test water (Table 12). The range of heterotrophic bacterial population density was from 6.0 to 7.5 $\times 10^6$ inds/mL in test water (Table 12). However, after ECS treatment, the number of living organisms for all groups in control and treated water met the D-2 regulation.

Table 11. Water quality of sea water (Masan Bay)

Date	Sample	pH	Temp. (℃)	Salinity (PSU)	DO Mg/L	TSS mg/L	DOC mg/L	POC Mg/L
2007.12.07	**Test water**	8.1	9.5	32.7	9.3	40.4	1.6	2.9
2007.12.12	**Control**	8.2	9.2	32.8	6.6	42.7	1.5	1.5
2007.12.12	**Treated**	8.3	8.9	32.8	8.8	34.3	2.1	1.3

Table 12. Biological efficacy of ECS from land-based test at sea water (Masan Bay)

Date	Sample	Sampling at	50µm< (inds/m^3)	10~50µm (inds/mL)	Heterotro. Bacteria (inds/mL)	*E. coli* (cfu/100 mL)	*Vibrio cholerae* (cfu/100 mL)	*Entero coccus* (cfu/100 mL)
2007.12.07	**Test water**	**20%**	233,492	1,652	6.0E+06	135	0	33
		50%	250,508	2,077	6.4E+06	148	0	33
		80%	267,733	1,608	7.5E+06	245	0	73
2007.12.12	**Control**	**20%**	6,612	229		25	0	5
		50%	7,956	174		30	0	5
		80%	9,202	244		45	0	10
2007.12.12	**Treated**	**20%**	0	0		0	0	0
		50%	0	0		0	0	0
		80%	0	0		0	0	0

2.5.4. S-4 (Sea water in Masan Bay)

The organism concentrations of test water for the land-based test sampling were set as the guideline of G8. The water quality of the location is summarized in Table 13. The range of the organism concentration for the group bigger than 50µm in size was from 291,738 to 368,588 inds/m^3, while that of the group between 10µm and 50µm in size was from 1,256 to 1,591 inds/mL in test water (Table 14). The range of heterotrophic bacterial population density was from 3.6 to 3.8 $\times 10^6$ inds/mL in test water (Table 14). However, after ECS treatment, the number of living organisms for all groups in control and treated water met the D-2 regulation.

Table 13. Water quality of sea water (Masan Bay)

Date	Sample	pH	Temp. (℃)	Salinity (PSU)	DO Mg/L	TSS mg/L	DOC mg/L	POC mg/L
2007.12.17	**Test water**	7.9	9.3	32.1	10.1	63.1	2.0	1.7
2007.12.22	**Control**	8.5	8.5	32.5	8.4	61.6	2.2	1.2
2007.12.22	**Treated**	8.3	8.5	32.6	9.5	59.9	3.1	1.0

Table 14. Biological efficacy of ECS from land-based test at sea water (Masan Bay)

Date	Sample	Sampling at	50µm< (inds/m^3)	10~50µm (inds/mL)	Heterotro. Bacteria (inds/mL)	*E. coli* (cfu/100 mL)	*Vibrio cholerae* (cfu/100 mL)	*Entero coccus* (cfu/100mL)
2007.12.17	**Test water**	**20%**	291,738	1,430	3.8E+06	119	0	163
		50%	329,250	1,591	3.6E+06	106	0	110
		80%	368,588	1,256	3.6E+06	110	0	108
2007.12.22	**Control**	**20%**	98,766	522		20	0	19
		50%	61,935	979		30	0	21
		80%	21,255	1,087		48	0	23
2007.12.22	**Treated**	**20%**	6	0			0	0
		50%	2	0			0	2
		80%	3	0			0	0

2.5.5. S-5 (Sea water in Masan Bay)

The organism concentrations of test water for the land-based test sampling were set as the guideline of G8. The water quality of the location is summarized in Table 15. The range of the organism concentration for the group bigger than 50µm in size was from 120,517 to 126,254 inds/m^3, while that of the group between 10µm and 50µm in size was from 1,450 to 1,693 inds/mL in test water (Table 16). The range of heterotrophic bacterial population density was from 1.4 to 1.8 $\times 10^6$ inds/mL in test water (Table 16). However, after ECS treatment, the number of living organisms for all groups in control and treated water met the D-2 regulation.

Table 15. Water quality of sea water (Masan Bay)

Date	Sample	pH	Temp. (℃)	Salinity (PSU)	DO mg/L	TSS mg/L	DOC mg/L	POC mg/L
2008.01.16	**Test water**	8.1	6.9	32.9	10.2	58.5	1.8	1.1
2008.01.21	**Control**	8.1	5.9	33.0	9.7	66.1	2.0	1.1
2008.01.21	**Treated**	8.2	5.9	33.0	10.4	60.7	1.8	0.5

Table 16. Biological efficacy of ECS from land-based test at sea water (Masan Bay)

Date	Sample	Sampling at	50µm< (inds/m^3)	10~50µm (inds/mL)	Heterotro. Bacteria (inds/mL)	*E. coli* (cfu/100 mL)	*Vibrio cholerae* (cfu/100mL)	*Enterococcus* (cfu/100mL)
2008.01.16	**Test water**	**20%**	120,517	1,450	1.4E+06	103	0	40
		50%	126,254	1,693	1.4E+06	106	0	61
		80%	122,454	1,469	1.8E+06	133	0	54
2008.01.21	**Control**	**20%**	3,900	135		12	0	38
		50%	2,511	151		42	0	30
		80%	1,739	192		15	0	15
2008.01.21	**Treated**	**20%**	3	0		0	0	3
		50%	3	0		0	0	5
		80%	1	0		0	0	3

2.5.6. B-1 (Brackish water in Sumjin River)

The organism concentrations of test water for the land-based test sampling were set following the guideline of G8. The water quality of the location is summarized in Table 17. The range of the organism concentration for the group bigger than 50μm in size was from 107,100 to 122,175 inds/m^3, while that of the group between 10μm and 50μm in size was from 1,114 to 1,320 inds/mL in test water (Table 18). The range of heterotrophic bacterial population was from 2.2 to 3.0 $\times 10^6$ inds/mL in test water (Table 18). However, after ECS treatment, the number of living organisms for all groups in control and treated water met the D-2 regulation.

Table 17. Water quality of sea water (Sumjin Estuary)

Date	Sample	pH	Temp. (℃)	Salinity (PSU)	DO mg/L	TSS mg/L	DOC mg/L	POC mg/L
2007.08.01	**Test water**	7.9	27.0	19.3	6.5	52.9	5.4	6.2
2007.08.06	**Control**	7.9	27.7	19.3	6.3	58.3	5.5	5.4
2007.08.06	**Treated**	7.9	27.0	19.3	7.5	65.7	5.7	5.4

Table 18. Biological efficacy of ECS from land-based test at sea water (Sumjin Estuary)

Date	Sample	Sampling at	50μm< (inds/m^3)	10~50μm (inds/mL)	Heterotro. Bacteria (inds/mL)	*E. coli* (cfu/100 mL)	*Vibrio cholerae* (cfu/100mL)	*Enterococcus* (cfu/100mL)
2007.08.01	**Test water**	**20%**	122,175	1,212	3.0E+06	823	0	30
		50%	121,058	1,114	2.2E+06	720	0	8
		80%	107,100	1,320	2.7E+06	653	0	18
2007.08.06	**Control**	**20%**	464	373		2	0	1
		50%	1210	480		3	0	8
		80%	1165	1,138		5	0	3
2007.08.06	**Treated**	**20%**	0	0		0	0	0
		50%	0	0		0	0	0
		80%	0	0		0	0	0

2.5.7. B-2 (Brackish water in Sumjin River)

The organism concentrations of test water for the land-based test sampling were set as the guideline of G8. The water quality of the location is summarized in Table 19. The range of the organism concentration for the group bigger than 50µm in size was from 234,421 to 244,642 inds/m^3, while that of the group between 10µm and 50µm in size was from 3,096 to 4,813 inds/mL in test water (Table 20). The range of heterotrophic bacterial population density was from 6.6 to 7.7 $\times 10^6$ inds/mL in test water (Table 20). However, after ECS treatment, the number of living organisms for all groups in control and treated water met the D-2 regulation.

Table 19. Water quality of sea water (Sumjin Estuary)

Date	Sample	pH	Temp. (℃)	Salinity (PSU)	DO mg/L	TSS mg/L	DOC Mg/L	POC mg/L
2007.08.22	**Test water**	8.3	27.9	18.2	8.5	60.3	6.7	6.0
2007.08.27	**Control**	8.0	29.0	18.2	5.3	62.6	5.5	5.7
2007.08.27	**Treated**	8.0	27.7	18.2	7.6	60.7	5.5	5.4

Table 20. Biological efficacy of ECS from land-based test at sea water (Sumjin Estuary)

Date	Sample	Sampling at	50µm< (inds/m^3)	10~50µm (inds/mL)	Heterotro. Bacteria (inds/mL)	*E. coli* (cfu/100mL)	*Vibrio cholerae* (cfu/100 mL)	*Entero coccus* (cfu/100mL)
2007.08.22	**Test water**	**20%**	244,642	4,258	7.7E+06	290	0	18
		50%	234,421	4,813	7.3E+06	278	0	16
		80%	237,383	3,096	6.6E+06	249	0	22
2007.08.27	**Control**	**20%**	8,438	206		2	0	33
		50%	5,256	240		5	0	0
		80%	6,146	732		5	0	0
2007.08.27	**Treated**	**20%**	0	0		0	0	0
		50%	0	0		0	0	0
		80%	0	0		0	0	0

2.5.8. B-3 (Brackish water in Sumjin River)

The organism concentrations of test water for the land-based test sampling were set as the guideline of G8. The water quality of the location is summarized in Table 21. The range of the organism concentration for the group bigger than 50μm in size was from 104,467 to 118,442 inds/m^3, while that of the group between 10μm and 50μm in size was from 2,025 to 2,277 inds/mL in test water (Table 22). The range of heterotrophic bacterial population density was from 2.5 to 3.0 $\times 10^6$ inds/mL in test water (Table 22). However, after ECS treatment, the number of living organisms for all groups in control and treated water met the D-2 regulation.

Table 21. Water quality of sea water (Sumjin Estuary)

Date	Sample	pH	Temp. (℃)	Salinity (PSU)	DO mg/L	TSS mg/L	DOC Mg/L	POC mg/L
2007.08.29	**Test water**	7.9	26.5	18.6	7.8	60.3	6.5	5.5
2007.09.03	**Control**	7.7	22.2	18.8	7.8	67.9	6.4	5.8
2007.09.03	**Treated**	7.9	21.5	18.6	9.1	64.1	6.6	5.3

Table 22. Biological efficacy of ECS from land-based test at sea water (Sumjin Estuary)

Date	Sample	Sampling at	50μm< (inds/m^3)	10~50μm (inds/mL)	Heterotro. Bacteria (inds/mL)	*E. coli* (cfu/100mL)	*Vibrio cholerae* (cfu/100 mL)	*Entero coccus* (cfu/100 mL)
2007.08.29	**Test water**	**20%**	108,508	2,211	2.9E+06	494	0	100
		50%	104,467	2,277	3.0E+06	501	0	75
		80%	118,442	2,025	2.5E+06	521	0	71
2007.09.03	**Control**	**20%**	1,968	134		135	0	110
		50%	1,703	88		168	0	61
		80%	2,634	89		87	0	63
2007.09.03	**Treated**	**20%**	0	0		0	0	0
		50%	0	0		0	0	0
		80%	0	0		0	0	0

2.5.9. B-4 (Brackish water in Sumjin River)

The organism concentrations of test water for the land-based test sampling were set as the guideline of G8. The water quality of the location is summarized in Table 23. The range of the organism concentration for the group bigger than 50µm in size was from 118,458 to 166,775 inds/m^3, while that of the group between 10µm and 50µm in size was from 1,554 to 2,131 inds/mL respectively, in test water (Table 24). The range of heterotrophic bacterial population density was from 3.8 to 4.4 $\times 10^6$ inds/mL in test water (Table 24). However, after ECS treatment, the number of living organisms for all groups in control and treated water met the D-2 regulation.

Table 23. Water quality of sea water (Sumjin Estuary)

Date	Sample	pH	Temp. (℃)	Salinity (PSU)	DO mg/L	TSS mg/L	DOC Mg/L	POC mg/L
2007.09.10	**Test water**	8.0	23.9	19.2	8.9	63.5	5.7	6.3
2007.09.15	**Control**	8.0	24.1	19.1	6.9	66.4	5.4	6.2
2007.09.15	**Treated**	8.1	24.0	19.2	8.4	63.4	5.5	5.9

Table 24. Biological efficacy of ECS from land-based test at sea water (Sumjin Estuary)

Date	Sample	Sampling at	50µm< (inds/m^3)	10~50µm (inds/mL)	Heterotro. Bacteria (inds/mL)	*E. coli* (cfu/100mL)	*Vibrio cholerae* (cfu/100 mL)	*Entero coccus* (cfu/100mL)
2007.09.10	**Test water**	**20%**	166,775	2,131	4.4E+06	837	0	525
		50%	150,704	1,808	4.2E+06	855	0	259
		80%	118,458	1,554	3.8E+06	663	0	298
2007.09.15	**Control**	**20%**	1,953	161		305	0	22
		50%	2,124	306		335	0	79
		80%	2,228	153		325	0	0
2007.09.15	**Treated**	**20%**	1	0		0	0	0
		50%	0	0		0	0	0
		80%	0	0		0	0	0

2.5.10. B-5 (Brackish water in Sumjin River)

The organism concentrations of test water for the land-based test sampling were set as the guideline of G8. The water quality of the location is summarized in Table 25. The range of the organism concentration for the group bigger than 50µm in size was from 108,858 to 116,100 inds/m^3, while that of the group between 10µm and 50µm in size was from 1,438 to 1,692 inds/mL in test water (Table 26). The range of heterotrophic bacterial population density was from 3.1 to 5.4 $\times 10^6$ inds/mL in test water (Table 26). However, after ECS treatment, the number of living organisms for all groups in control and treated water met the D-2 regulation.

Table 25. Water quality of sea water (Sumjin Estuary)

Date	Sample	pH	Temp. (℃)	Salinity (PSU)	DO mg/L	TSS mg/L	DOC Mg/L	POC mg/L
2007.10.22	**Test water**	8.0	21.6	20.1	19.1	57.9	6.1	5.8
2007.10.27	**Control**	8.0	21.0	20.3	16.1	58.9	6.1	5.9
2007.10.27	**Treated**	8.0	20.7	20.0	17.1	62.2	5.7	5.7

Table 26. Biological efficacy of ECS from land-based test at sea water (Sumjin Estuary)

Date	Sample	Sampling at	50µm< (inds/m^3)	10~50µm (inds/mL)	Heterotro. Bacteria (inds/mL)	*E. coli* (cfu/100 mL)	*Vibrio cholerae* (cfu/100 mL)	*Entero coccus* (cfu/100 mL)
2007.10.22	**Test water**	**20%**	116,100	1,498	5.4E+06	837	0	525
		50%	108,858	1,438	3.1E+06	855	0	259
		80%	114,471	1,692	4.7E+06	663	0	298
2007.10.27	**Control**	**20%**	1,167	143		277	0	55
		50%	1,633	135		267	0	62
		80%	1,338	236		277	0	179
2007.10.27	**Treated**	**20%**	0	0		0	0	0
		50%	0	0		0	0	0
		80%	0	0		0	0	0

2.5.11. B-6 (Brackish water in Sumjin River)

The organism concentrations of test water for the land-based test sampling were set as the guideline of G8. The water quality of the location is summarized in Table 27. The range of the organism concentration for the group bigger than 50µm in size was from 201,025 to 275,420 inds/m^3, while that of the group between 10µm and 50µm in size was from 2,720 to 3,613 inds/mL in test water (Table 28). The range of heterotrophic bacterial population density was from 7.0 to 26 $\times 10^5$ inds/mL in test water (Table 28). However, after ECS treatment, the number of living organisms for all groups in control and treated water met the D-2 regulation.

Table 27. Water quality of sea water (Sumjin Estuary)

Date	Sample	pH	Temp. (℃)	Salinity (PSU)	DO mg/L	TSS mg/L	DOC Mg/L	POC mg/L
2008.02.20	**Test water**	8.5	5.9	16.8	11.7	59.5	5.4	6.1
2008.02.25	**Control**	8.1	5.4	17.3	9.8	53.9	5.3	6.5
2008.02.25	**Treated**	8.5	5.1	17.0	11.5	52.2	5.9	6.3

Table 28. Biological efficacy of ECS from land-based test at sea water (Sumjin Estuary)

Date	Sample	Sampling at	50µm< (inds/m^3)	10~50µm (inds/mL)	Heterotro. Bacteria (inds/mL)	*E. coli* (cfu/100 mL)	*Vibrio cholerae* (cfu/100 mL)	*Entero coccus* (cfu/100 mL)
2008.02.20	**Test water**	**20%**	275,420	2,878	2.6E+06	5	0	4
		50%	201,025	2,720	7.8E+05	2	0	3
		80%	227,113	3,613	7.0E+05	0	0	2
2008.02.25	**Control**	**20%**	36,400	435		2	0	5
		50%	30,657	412		0	0	4
		80%	7,556	463		0	0	13
2008.02.25	**Treated**	**20%**	0	0		0	0	0
		50%	0	0		0	0	0
		80%	0	0		0	0	0

3. Shipboard test

Shipboard test was performed by M/V STX YOKOHAMA, based on the D-2 regulation. Test done by M/V STX YOKOHAMA was performed to prove the efficacy of sea water disinfection, and the test performed was to prove the efficacy of sea water disinfection. All tests were performed at the Korea Marine Research Center, and satisfied D-2 regulation.

Shipboard test by M/V STX YOKOHAMA have been performed every one week in different seas (Korea, China, and Japan) from Feb 3rd. 2007 till Jul 30th 2007 (Total of 24weeks).

The operation conditions of ECS was adjusted to generate 10mg/L of TRO, where the conditions of electric current and voltage were regulated according to the condition of water quality of test during in-ballasting by the ship (salinity, temperature, and organic matter contents *etc*.). Continuous tests of section 8 were successful and satisfied D-2 standard.

The procedures and sampling diagram for shipboard test are summarized in Fig. 9 and 10.

- Sampling for source, treated, and control water to be collected and tested.
- For each water, sampling from three different phases to be executed (10%, 50% and 80% of full ballast tanks).
- The above procedure is consistent with D-2 and G-8 sampling protocol of the Ballast Water Management Convention.

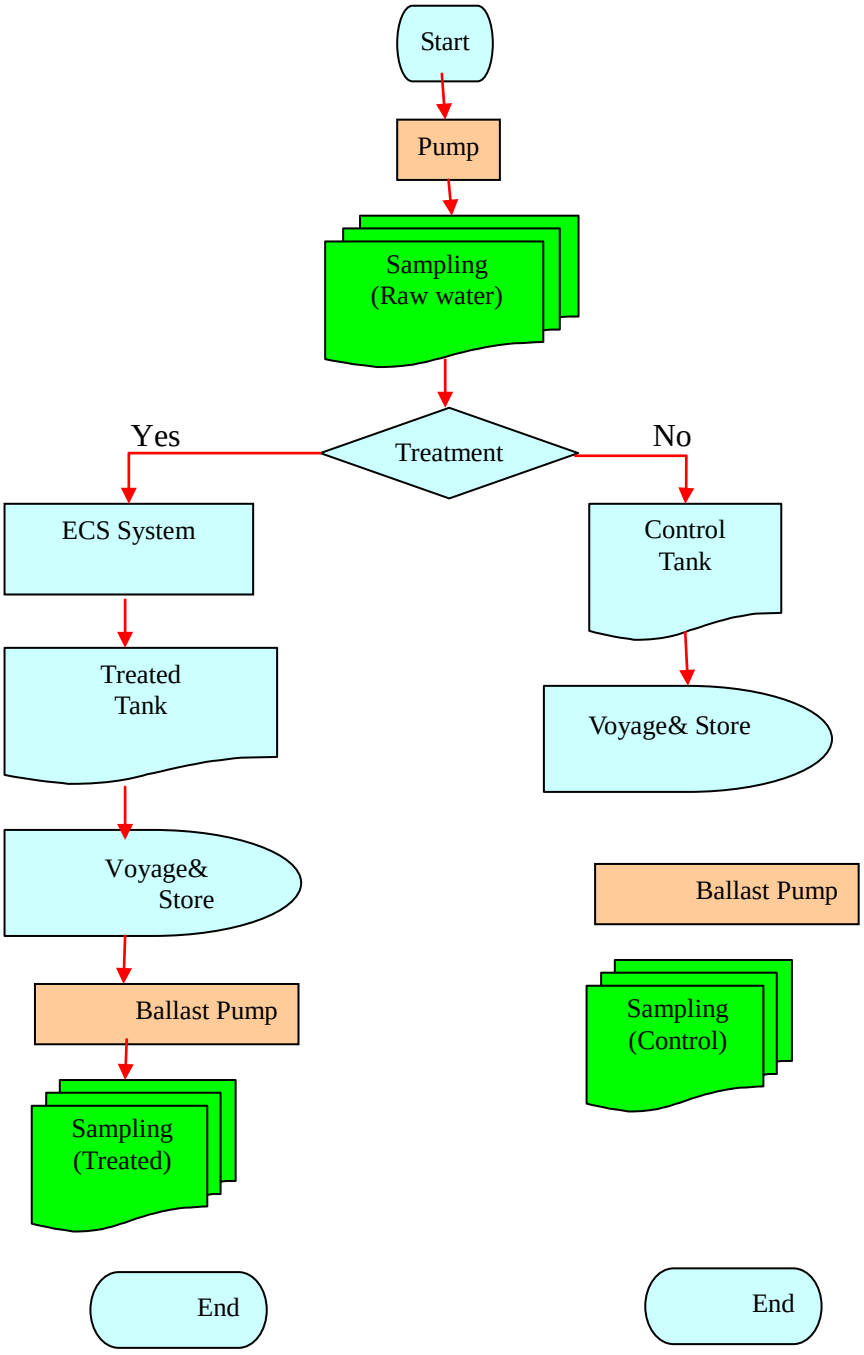
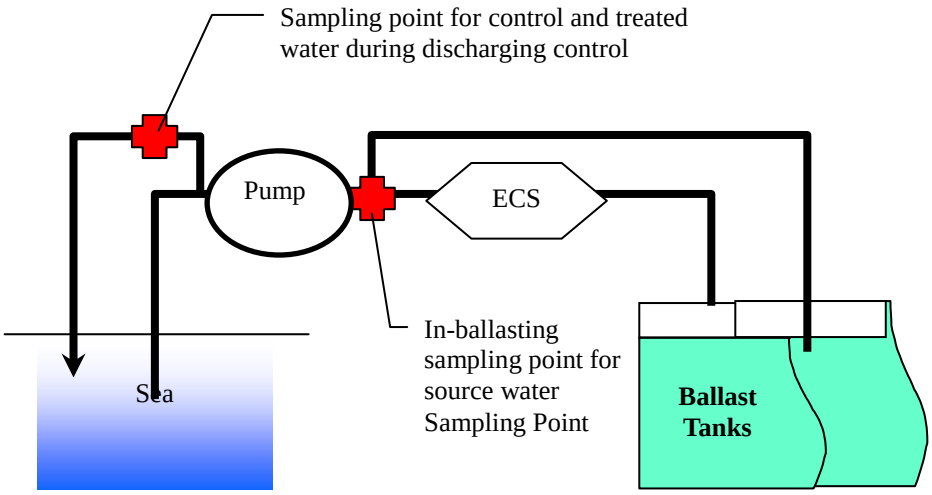


Fig. 9. Procedure of shipboard test



※ The sampling point for the source water is directly after the ballast pump and for control and treated water is where the sea water has passed the ballast pump twice.

Fig. 10. Sampling diagram

3.1. M/V STX YOKOHAMA

3.1.1. Information of the test vessel

The specification and main instrument of M/V STX YOKOHAMA for shipboard test is summarized in Table 21 and 22, respectively.

Table 21. M/V STX YOKOHAMA specifications

Component	**Specifications**
Name / type	M/V STX YOKOHAMA / Container ship
Gross tonnage	8,306m^3
Dimensions	Length: 132.94m Width: 20.50m Depth: 10.50m
Owner	STX PAN OCEAN
Builder	HANJIN
Launching Date	May. 30.1998

Table 22. M/V STX YOKOHAMA main instruments

Component	Description
Pipe and pump	Maximum amount to pump: 600m^3/h (300m^3/h for 2 ships)
Dark condition tank	Actual employed ballast water tank
Equipment for collecting samples	Netting for collecting proper amount of micro-organisms Net for collecting samples
Equipment for micro-organism test	Pump placed underwater for collecting samples refrigerator for storing samples (4℃)
Equipment for running instrument	Use power supply for driving ships
Ballast management system	ECS-300A 1 set
Repair tools	Reserved stocks and tools for repairing instrument
Extra equipment	Living facility for researcher and inspector (Restaurant, bedroom, conference room)

3.1.2. Test sites

The travel course and test site for shipboard test with M/V STX YOKOHAMA were as follows (Table 23 and Figs. 11 and 12):

- Travel courses of M/V STX YOKOHAMA are 47, 48 and 49. The oceanic ecosystem between these courses is very different.
- M/V STX YOKOHAMA sails the Japanese and Chinese courses once per week.
- Course of Japan (one week): Busan → Yokohama → Tokyo → Shimizu → Nagoya → Yokaichi → Busan
- Course of China (one week): Busan → Masan → Kwangyang → Qingdao→ Busan
- Course of Korea (5 days): Busan → Kwangyang

Table 23. Sea area of navigation

Country	Anchoring port	Sea area (LME Model No.)
Korea	Busan port, Masan port, Kwangyang port	East China Sea (47)
China	Qingdao port	Yellow Sea (48)
Japan	Nagoya port, Tokyo port, Yokohama port, Shimizu port	Kuroshio Current (49)

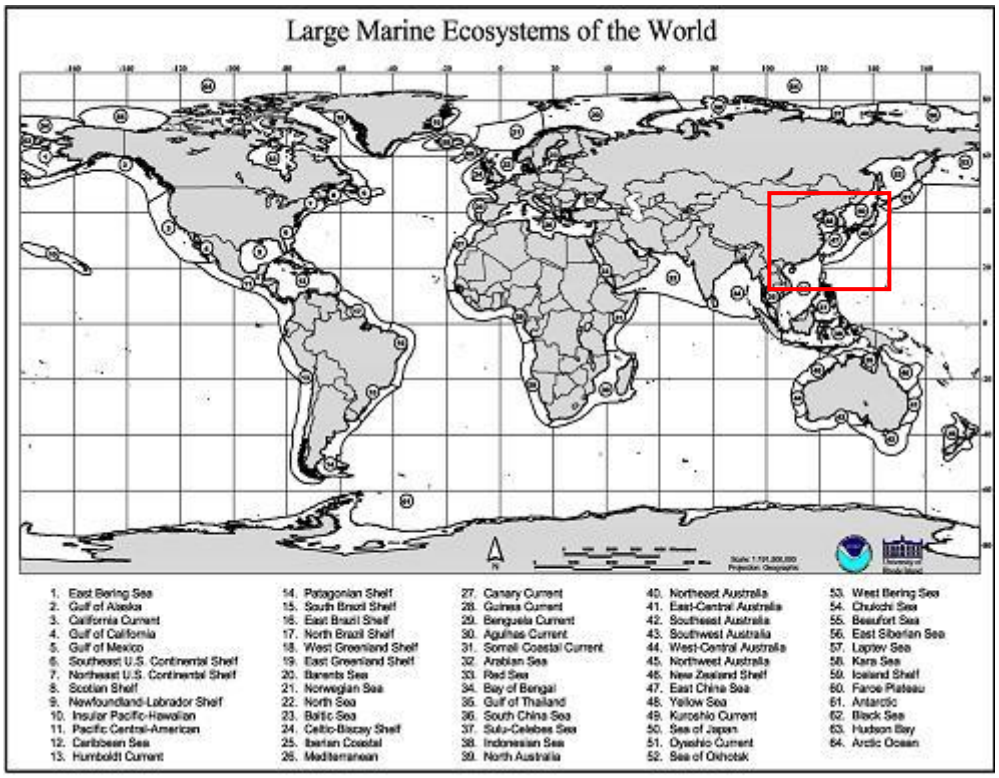


Fig. 11. Navigation map of M/V STX YOKOHAMA

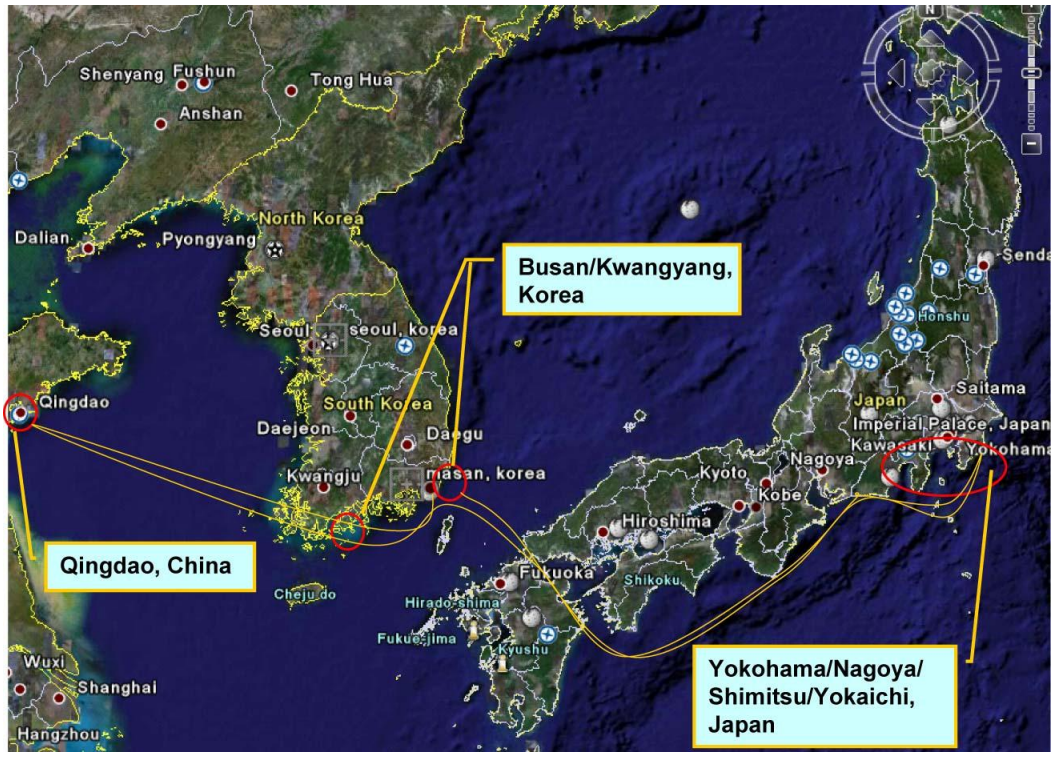


Fig. 12. Navigational sea areas of M/V STX YOKOHAMA

3.1.3. Installation for the test

The biological efficacy test system of ECS for shipboard test at M/V STX YOKOHAMA were composed of: ECS-300A instrument, 4 tanks with volume of 250m^3 each and each of the pipes and valves were designed to fit to regulation. The rate at which a ballast pump can take up sea water is 300m^3/h.

Plankton net for sampling was installed through the path of the in-ballasting pipeline, and controlled water and treated water were sampled based on the guidelines of shipboard test. Flow meter was installed to measure the amount of sampled water and it is managed to control the flow rate of 1m^3 every 10minutes.

3.1.4. Preparation for the test

3.1.4.1. Cleaning test instruments

The ballast tank for the M/V STX YOKOHAMA was repeatedly emptied and filled ballast water as much as possible within the port so as to remove any of the remaining organic matter and sediments.

3.1.4.2. Test instruments

If any pollutants come in contact with the rectifier, distributor, control pc, or any other equipment, they need to be cleaned with a dry cloth and any rusty screws need to be replaced. Any equipment that requires a new air filter needs to have it replaced. Any connection cable parts in bad condition (rusty) need to be replaced before the test.

3.1.5. Test methods

3.1.5.1. Temperature and salinity

Temperature and salinity were measured using an Idronaut Ocean Seven 319 CTD. The equipment was calibrated once a year by the manufacturer.

3.1.5.2. pH

A pH meter used was a model 1230, manufactured by Orion Research Inc. The equipment was calibrated using standard solutions (Orion application buffer, pH 4, 7, 10) each time prior to the measurement according to the guidance provided by manufacturer.

3.1.5.3. Dissolved oxygen

DO was measured using an Idronaut Ocean Seven 319 CTD. The DO meter attached to the CTD was calibrated each time prior to the measurement according to the guidance provided by manufacturer. Maintenance (mostly membrane and electrolyte replacement) should be carried out at least every three months.

3.1.5.4. Determination of dissolved organic carbon (UNESCO, 1994)

For dissolved organic carbon (DOC) analysis, each seawater sample was filtered through a pre-combusted (450 °C, 2hours) 25mm Whattman GF/F filters (nominal pore size 0.7 μm) and 20 mL of filtered sample was collected in a pre-combusted 30-mL EPA vial (Wheaton, WH.W227354). After collection, the filtered seawater sample was immediately acidified with 10% H3PO4 solution and purged with ultrapure O_2 gas for 10 min to remove dissolved inorganic carbon (DIC). One hundred microliters of the DIC-free subsample was then injected into the combustion tube of a Shimadzu TOC-VCPH total organic carbon analyzer for the oxidation of DOC to CO_2, which was facilitated by a platinum catalyst at 650 °C. The liberated CO_2 was subsequently measured using an infrared detector. On each day of measurements, a three-point calibration curve was constructed using potassium phthalate standards freshly prepared in Milli-Q water. These standards covered a DOC concentration ranged of 0 to 10 mg/L and were run once a day. All DOC measurements reported here represent the mean of three injections from each sample.

3.1.5.5. Determination of dissolved particulate organic carbon (UNESCO, 1994)

For determination of dissolved particulate organic carbon (POC), samples were collected in 2 liter sterilization bottles. 200 mL of seawater sample was filtered onto pre-combusted (450 °C, 2hours) 25mm Whattman GF/F filters (nominal pore size 0.7 μm). During filtration, a slight vacuum (0.0027 MPa or 200 torr) was applied to avoid rupture of the cells on the filters. After filtration, the wet filters were dislodged from the filter holders and then stored frozen in a deep freezer (-20 °C) until processed. Prior to analysis, the filters are placed overnight in a desiccator saturated with HCl fumes. The air in the desiccator is kept saturated by leaving concentrated HCl in an open container in the lower compartment of the desiccator. Thereafter, the filters are dried again at 65 °C for two days. Immediately before analysis, with the use of a clean pair of forceps, the dried filters were folded with tinfoil and palletized (Sharp, 1974). The CHN elemental analyzer (ThermoQuest, EA1112) was used to determine the concentration of POC on the dried filters.

3.1.5.6. Determination of total suspended substance (APHA, 1995)

Whattman GF/F filters (47mm, nominal pore size 0.7 μm) were rinsed with 20mL of distilled water three times and dried in the oven at 103 to 105 °C for 1 hour and then cooled to the room temperature for 30 minutes in the desiccator. The filters were then weighted. The sample volumes were chosen for the sufficient amount to yield residue between 10 mg and 200 mg after filtration. When there were very low total solids in samples (less than 10 mg/L), the deficiency of low weight was compensated by using a high-sensitive balance (0.001 mg). The appropriate amount of sample was filtered through the prepared 47mm Whatman GF/F filter and the residue retained on the filter was dried at 103 to 105 °C for 1 hour. The filter was cooled in desiccator to room temperature for 30 minutes, and then re-weighted. The difference between pre and after weighted filter was divided by sample volume (unit liter) and represents the concentration of total suspended solid.

3.1.5.7. Viability test of organisms

- **Organisms ≥ 10~50μm**

  The disinfection efficacy of the ECS on 10~50μm sized organisms (mainly phytoplankton) was assessed by three kinds of measurements using photomicroscope, epifluorescence microscope and fluorometer (Turner Designs 10-AU).

  With the use of the light microscope, the motility; for example of sliding or its own original movement assessed vitality of the phytoplankton.

  Chlorophyll autofluorescence is used as an indicator of cell viability (Pouneva, 1997). Intact chlorophyll of living cells shows red fluorescence, while dead or severely damaged chlorophyll lose the red fluorescence. Commonly, under a green filter using an epifluorescence microscope, the most living cells shows brightly red colour, while dead cells show faintly green colour or disappearance of red fluorescence.

  Fluorometer with high sensitivity can detect the fluorescence of phytoplankton more than one live cell per milliliter. Thus, if no value is read on the display of fluorometer, all cells are considered to be ‘dead’, while if any number is read; there are live cells in the sample. Fluorometer was used as a supplementary means.

- **Organisms > 50µm**

Survivorship of the larger organisms 50µm (mainly zooplankton) was determined based on the appendage's movement under a stereomicroscope (APHA-804C, 1985). In each taxonomic group, individuals were classified as live or dead and counts of each group were recorded. Animals were considered to be 'live' if they are actively moving or exhibited an escape behavior when probed with a fine needle. If no activity or movement of any kind was observed, after the additional sticking with a fine needle, the animals were considered to be 'dead'. And the determination of live or dead is confined to the unimpaired body of zooplankton. Abovementioned assessment method was also applied to the non-motile organisms larger than 50 µm.

- **DAPI count for heterotrophic bacteria**

For setting up the condition of influent water, 50mL seawater was fixed with neutralized formalin (final conc. 2~4%). One millilitre in well-fixed seawater is stained with 0.2mL DAPI (6-diamidino-2-phenylindole) working solution. After 5 minutes, at least 10 fields were counted under UV filter set of fluorescent microscopy at (× 1,000).

- **Indicator microbes**

• ***Escherichia coli* (*E. coli*)**

A 10mL of sea water was filtered onto the 0.2µm membrane filter and then filters are placed on the top of *E. coli*/ Coliform and Coliform Count Plates (3M^{TM} Petrifilm plate). The Petrifilm plates were incubated for 24 hrs at 35℃. The blue to red-blue colonies associated with entrapped gas in the Petrifilm EC plate (within approximately one colony diameter) is considered as *E. coli*. The enumeration of each sample was repeated eight to ten times.

• ***Vibrio cholerae* (serotypes O1 and O139)**

5 to 10 mL of sea water was filtered onto the 0.2µm membrane filter and then filters were placed on the TCBS (Bisulfate Citrate Bile Sucrose) agar. Pre-treated TCBS agar plates were incubated for 24 hrs at 35℃. The green coloured CFU is considered as *Vibrio parahaemolyticus*. The yellow coloured CFU is isolated into nutrient agar and incubated for 24 hrs at 35℃. If the cultivated CFU has purple colour, it is considered to be positive, if not or partly purple, it is negative. When the incubated CFU is determined

to be positive, API20E test should be carried out. The possibility of *Vibrio cholerae* in the case of partly purple coloured CFU was tested, but any CFU was not found in the API20E test. The enumeration of each sample was repeated eight to ten times.

• ***Enterococci***

A 20 ~ 40 mL of sea water was filtered onto the 0.2μm membrane filter and then filters are placed on the Intestinal *Enterococci* agar plate. The pre-treated agar plates were incubated for 48 hrs at 35℃. The pink to brown coloured CFU with a diameter of 0.5 to 2 mm is usually *Enterococci*. The enumeration of each sample was repeated eight to ten times.

Finally, the above mentioned test was additionally conducted with disinfected distilled water in order to confirm whether or not there is any contamination as a control. The levels of organic matters in test sites for shipboard test are summarized in table 24.

Table 24. A brief summary of water qualities at the test sites for shipboard test on M/V STX YOKOHAMA

Salinity: 30~32PSU

	S-1	**S-2**	**S-3**
DOC (mg/L)	1.66	1.58	1.03
POC (mg/L)	0.85	0.88	0.44
TSS (mg/L)	47.4	71.4	64.9

3.1.6. Test results

The shipboard tests that were performed 3 times satisfied D-2 regulation for disinfection of each size of micro-organisms.

3.1.6.1. S-1 (M/V STX YOKOHAMA)

The water quality at the point of the shipboard test is summarized in Table 25. The range of the organism concentration for the group bigger than 50µm in size was from 3,703 to 5,793 inds/m^3, while that of the group between 10µm and 50µm in size was from 1,963 to 2,521 inds/mL respectively, in raw water (Table 26). After voyage, the range of living organism concentration for the group bigger than 50µm in size was 270 to 371 inds/ m^3, while that of the group between 10µm and 50µm in size was from 12 to 15 inds/mL respectively, control water. There were no living organisms found in treated water.

Table 25. Water quality for shipboard test

Date	Sample	pH	Temp. (°C)	Salinity (PSU)	DO mg/L	TSS mg/L	DOC mg/L	POC mg/L
2007.07.03	**Raw water**	7.8	23.1	29.0	-	47.4	1.66	0.85
2007.07.07	**Control**	7.7	23.1	29.4	5.2	38.4	1.42	0.3
2007.07.07	**Treated**	8.0	23.2	28.9	5.0	44.1	1.56	0.4

Table 26. Biological efficacy of ECS from shipboard test

Date	Sample	Sampling at	50µm< (inds/m^3)	10~50µm (inds/mL)	Heterotro. Bacteria (inds/mL)	*E. coli* (cfu/100mL)	*Vibrio cholerae* (cfu/100 mL)	*Entero coccus* (cfu/100 mL)
2007.07.03	**Raw water**	**20%**	4,917	2,160	2.9 E+06	85	0	7
		50%	5,739	2,521	3.0 E+06	234	0	26
		80%	3,703	1,936	6.0 E+06	260	0	26
2007.07.07	**Control**	**20%**	270	12	-	30	0	5
		50%	371	15	-	20	0	0
		80%	282	12	-	35	0	0
2007.07.07	**Treated**	**20%**	0	0	-	0	0	0
		50%	0	0	-	0	0	0
		80%	0	0	-	0	0	0

3.1.6.2. S-2 (M/V STX YOKOHAMA)

The water quality at the point of the shipboard test is summarized in Table 27. The range of the organism concentration for the group bigger than 50μm in size was from 3,522 to 8,072 inds/m^3, while that of the group between 10μm and 50μm in size was from 1,316 to 2,016 inds/mL respectively, in raw water (Table 28). After voyage, the range of living organism concentration for the group bigger than 50μm in size was 811 to 920 inds/ m^3, while that of the group between 10μm and 50μm in size was from 9 to 16 inds/mL respectively, control water. There were no living organisms found in treated water.

Table 27. Water quality for shipboard test

Date	Sample	pH	Temp. (°C)	Salinity (PSU)	DO mg/L	TSS mg/L	DOC mg/L	POC mg/L
2007.07.11	**Raw water**	8.1	24.4	31.0	-	71.4	1.58	0.88
2007.07.15	**Control**	7.4	22.2	31.4	7.6	46.9	1.50	0.46
2007.07.15	**Treated**	7.6	22.1	31.4	7.5	55.7	1.84	0.35

Table 28. Biological efficacy of ECS from shipboard test

Date	Sample	Sampling at	50μm< (inds/m^3)	10~50μm (inds/mL)	Heterotro. Bacteria (inds/mL)	*E. coli* (cfu/100mL)	*Vibrio cholerae* (cfu/100 mL)	*Entero coccus* (cfu/100 mL)
2007.07.11	**Raw water**	**20%**	3,522	920	6.5 E+06	20	0	0
		50%	8,072	811	6.2 E+06	39	0	0
		80%	7,071	816	5.6 E+06	39	0	0
2007.07.15	**Control**	**20%**	2,016	9	-	520	0	0
		50%	1,341	16	-	45	0	20
		80%	1,316	11	-	100	0	0
2007.07.15	**Treated**	**20%**	0	0	-	0	0	0
		50%	0	0	-	0	0	0
		80%	0	0	-	0	0	0

3.1.6.3. S-3 (M/V STX YOKOHAMA)

The water quality at the point of the shipboard test is summarized in Table 29. The range of the organism concentration for the group bigger than 50µm in size was from 66,684 to 100,241 inds/m^3, while that of the group between 10µm and 50µm in size was from 371 to 558 inds/mL respectively, in raw water (Table 30). After voyage, the range of living organism concentration for the group bigger than 50µm in size was 0 to 3,876 inds/ m^3, while that of the group between 10µm and 50µm in size was from 82 to 1,756 inds/mL respectively, control water. There were no living organisms in treated water.

Table 29. Water quality for shipboard test

Date	Sample	pH	Temp. (℃)	Salinity (PSU)	DO mg/L	TSS mg/L	DOC mg/L	POC mg/L
2007.07.22	**Raw water**	8.1	25	30	-	64.9	1.03	0.44
2007.07.23	**Control**	8	23.8	32.2	7.6	65.3	1.07	0.35
2007.07.23	**Treated**	8.5	23.3	33.2	5.8	62.5	1.06	0.28

Table 30. Biological efficacy of ECS from shipboard test

Date	Sample	Sampling at	50µm< (inds/m^3)	10~50µm (inds/mL)	Heterotro. Bacteria (inds/mL)	*E. coli* (cfu/100mL)	*Vibrio cholerae* (cfu/100 mL)	*Entero coccus* (cfu/100 mL)
2007.07.22	**Raw water**	**20%**	86,412	481	6.6E+06	416	0	0
		50%	100,241	558	8.0E+06	364	0	7
		80%	66,684	371	6.3E+06	611	0	7
2007.07.23	**Control**	**20%**	0	1,756	-	165	0	5
		50%	3,876	82	-	125	0	0
		80%	3,395	103	-	170	0	5
2007.07.23	**Treated**	**20%**	0	0	-	0	0	0
		50%	0	0	-	0	0	0
		80%	0	0	-	0	0	0

References

Pouneva, I., 1997. Evaluation of algal culture viability and physiological state by fluorescent microscopic methods. *Bulg. J. Plant Physiol*., 23(1-2): 67-76

Garvey, M., B. Moriceau, and U. Passow1, 2007. Applicability of the FDA assay to determine the viability of marine phytoplankton under different environmental conditions. *Marine Ecology Progress Series*, 352, 17-26

Jochem, F., 1999. Dark survival strategies in marine phytoplankton assessed by cytometric measurement of metabolic activity with fluorescin diacetate. *Mar Biol*, 135, 721–728

Sharp, J.H., 1974. Improved analysis for "particulate" organic carbon and nitrogen from seawater. Limnol. Oceanogr., 19: 984-989.

UNESCO, 1994. Protocols for Joint Global Carbon Flux Study (JGOFS) Core Measurement. Manuals and Guides, 29: 101-118 pp.

American Public Health Association (APHA), 1995. Standard Method for Examination of Water and Wastewater. American Water Association and Water Environment Federation 19th Edition Washington D.C., 2-56 pp.

American Public Health Association (APHA), 1985. Standard Method for Examination of Water and Wastewater. American Water Association and Water Environment Federation 16th Edition Washington D.C., 742-748 pp.

Annex : Certificate of Accreditation

Annex 1 : Certificate of Accreditation for ISO/IEC 17025

Name of Laboratory :	Korea Ocean Research and Development Institute (KORDI)
Date :	11th June 2007
Administrator :	Korea Laboratory Accreditation Scheme (KOLAS) Governmental accreditation body in the Republic of Korea

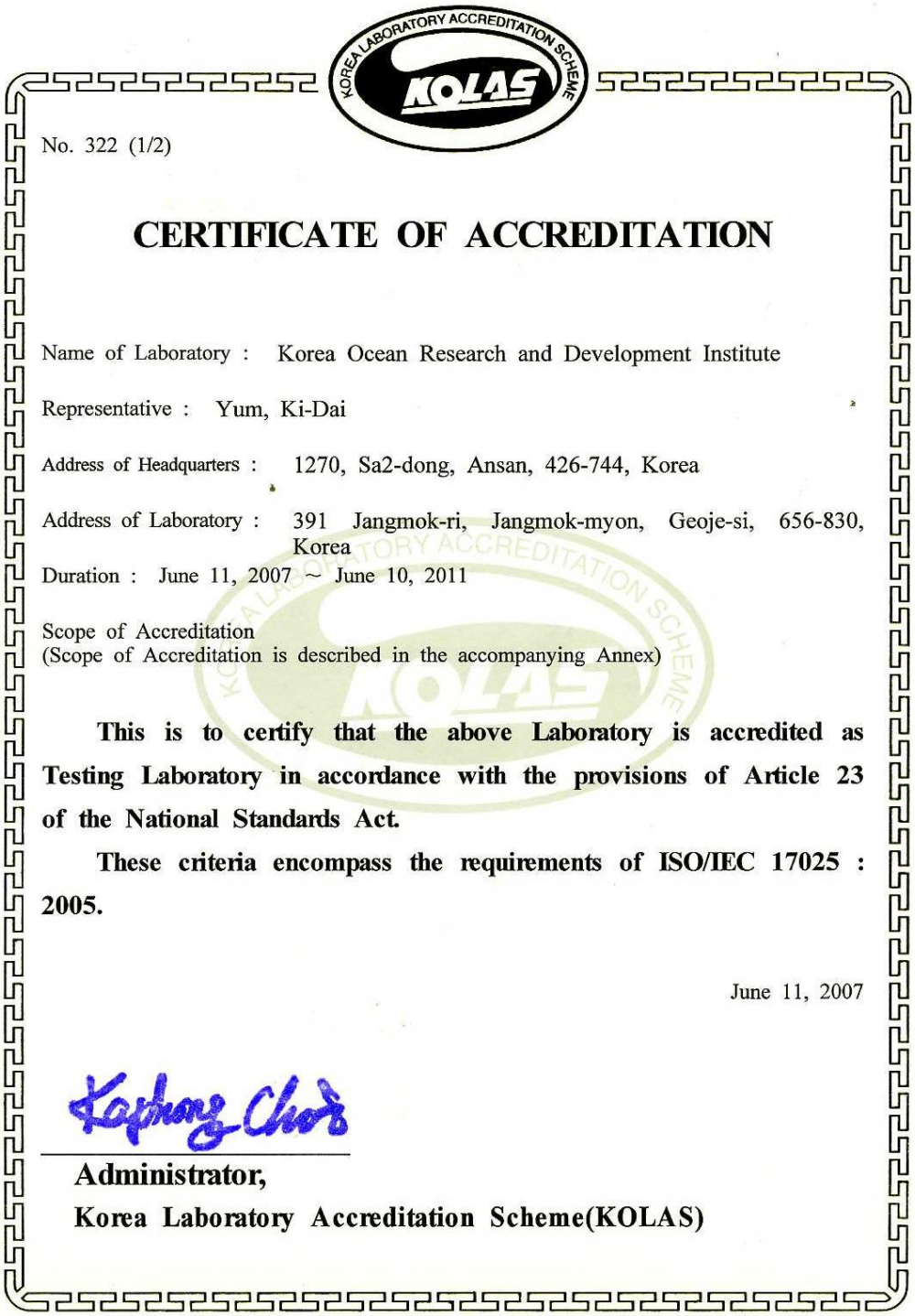
※ The ISO/IEC 17025 System were executed from October 2006 at KORDI in field of bio-efficacy test for type approval test of ballast water management system.

Annex 2 : Designation of Type Approval Testing Body

Name of Laboratory :	Korea Ocean Research and Development Institute (KORDI)
Date :	29th August 2007
Administrator :	Ministry of Maritime Affairs & Fisheries (MOMAF)
Regulation :	Provisional Regulation of Type Approval of Ballast Water Management System (2006-77, MOMAF, 8th November 2006)

※ According to the retroactive to Provisional Regulation of Type Approval of Ballast Water Management System (2006-77, MOMAF), the test results which conducted before designation identified as type approval test.

Annex 1 : Certificate of Accreditation for ISO/IEC 17025



No. 322 (1/2)

CERTIFICATE OF ACCREDITATION

Name of Laboratory : Korea Ocean Research and Development Institute

Representative : Yum, Ki-Dai

Address of Headquarters : 1270, Sa2-dong, Ansan, 426-744, Korea

Address of Laboratory : 391 Jangmok-ri, Jangmok-myon, Geoje-si, 656-830, Korea

Duration : June 11, 2007 ~ June 10, 2011

Scope of Accreditation
(Scope of Accreditation is described in the accompanying Annex)

This is to certify that the above Laboratory is accredited as Testing Laboratory in accordance with the provisions of Article 23 of the National Standards Act.

These criteria encompass the requirements of ISO/IEC 17025 : 2005.

June 11, 2007

Administrator,
Korea Laboratory Accreditation Scheme(KOLAS)

No. 322 (2/2)

9. Biological testing

9.006 Aquatic biology

Test method	Standard designation
APHA-804C : 1985	American Public Health Association (APHA)/Standard methods for the examination of water and wastewater/To ascertain if a motionless animal is dead, touch it gently with a sealed glass capillary probe
EPA-445.0 : 1997	Environmental Protection Agency (EPA)/In vitro determination of chlorophyll-*a* and pheophytin *a* in marine and freshwater algae by fluorescence

End.

Annex 2 : Designation of Type Approval Testing Body (Korean)

'국민참여가 나라를 바꿉니다'

해 양 수 산 부

수신자 한국해양연구원장 염기대
(경유)

제목 밸러스트수관리시스템 형식승인시험기관 지정

1. 귀원생태기능 제07-0040-02(2007.7.18)의 관련입니다.

2. 귀 원에서 신청한 밸러스트수관리시스템에 대한 형식승인시험(육상시험 및 선상시험) 지정에 대하여 내용 검토한 바, 「밸러스트수관리시스템의 형식승인 등에 관한 잠정기준, 고시 제2006-77호, 2006년 11월 8일」 제6조 제3항에 따른 지정기준을 만족하고 있어 아래의 조건으로 귀 원을 육상시험 및 선상시험기관으로 붙임과 같이 지정하고 통보 하오니 형식승인 업무에 철저를 기하여 주시기 바랍니다.

--- 아 래 ---

가. 인간건강 기준 관련시험은 자체 품질관리체제를 구축하여 국제적으로 공인된 시험방법 및 절차에 따라 실시할 것

나. 인간건강 기준 관련시험에 대하여 2008년까지 ISO/IEC KS 17025에 의한 인정요건을 충족할 것

붙임 형식승인시험기관 지정서_한국해양연구원_01. 끝.

해 양 수 산 부 장

08/29

주무관 김철홍 해양수산사무관 이기상 해사기술팀장 김삼열

협조자

시행 해사기술팀-1443 (2007.08.29) 접수 ()

우 110-793 서울시 종로구 계동 140-2, 현대빌딩 8층 / www.momaf.go.kr

전화 02-3674-6322 전송 02-3674-6327 / kch0928@momaf.go.kr / 공개

지정번호 제1호
Cert. No. 1

밸러스트수관리시스템 형식승인시험기관 지정서

Certificate of Accreditation as the Type Approval Test Organization of Ballast Water Management System

지정을 받는 자 Nominee	①명 칭(상 호) Name of Organization	한국해양연구원 Korea Ocean Research & Development Institute		
	②성 명(대표자) Representative	염기대 Yum, Ki-Dai	③주민등록번호 (법인등록번호) ID No	490906-1000525 (130122-0002126)
	④주 소(사업장) Address	대한민국 경기도 안산시 사동 1270 1270, Sa-dong, Ansan-si, Gyonggi-do, Korea (경상남도 거제시 장목면 장목리 391번지, 391, Jangmok-ri, Jangmok-myon, Geoje-si, Korea)		
⑤형식승인시험의 종류 Scope of Accreditation		육상시험 및 선상시험 Land-based testing and shipboard testing		

「밸러스트수관리시스템의 형식승인 등에 관한 잠정기준」제6조제3항에 따라 이 지정서를 교부합니다.

This is to certify that the above Body is accredited as a Type Approval Test Organization in accordance with the Interim Regulation for Type Approval of Ballast Water Management System and IMO MEPC Res. 125(53).

2007년 8월 29일
August 29, 2007

해 양 수 산 부 장 관
Minister of Ministry of Maritime Affairs and Fisheries